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# Modeling the effect of oregano essential oil on shelf-life extension of vacuum-packed cooked sliced ham

Natielle Maria Costa Menezes<sup>a</sup>, Wiaslan Figueiredo Martins<sup>a</sup>, Daniel Angelo Longhi<sup>b</sup>, Gláucia Maria Falcão de Aragão<sup>a,\*</sup>

<sup>a</sup> Federal University of Santa Catarina, Department of Chemical Engineering and Food Engineering, Center of Technology, Florianópolis, SC 88040-901, Brazil <sup>b</sup> Federal University of Paraná, Food Engineering, Campus Jandaia do Sul, Jandaia do Sul, PR 86900-000, Brazil

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# ABSTRACT

The present study modeled the effect of oregano essential oil, as an antimicrobial agent, on the shelf-life of vacuum-packed cooked sliced ham, based on the growth of lactic acid bacteria natural microbiota under isothermal conditions. The bacterial growth in ham without oregano essential oil (control) and with 0.4% oregano essential oil (v/w) was evaluated at five different temperatures (6, 12, 15, 20 and 25 °C). Baranyi and Roberts, and modified Gompertz primary models were fitted to microbial growth curves. Square Root and Exponential secondary models were fitted to  $\mu_{max}$  parameter data. The addition of oregano essential oil increased lag phase, decreased growth rates and extended shelf-life of ham for all temperatures (at 6 °C extended for, at least, 30 days when compared to control). Statistical indexes showed that Baranyi and Roberts, and Exponential were the primary and secondary models, respectively, that better fit to the data. Thus, oregano essential oil showed a good antimicrobial effect and extended the ham shelf-life.

### 1. Introduction

Lactic acid bacteria (LAB) are the major bacterial group associated with the spoilage of cooked meat products packed in vacuum and kept under refrigeration, including ham (Duskova, Kameník, Lacanin, Sedo, & Zdráhal, 2016; Samelis, Kakouri, & Rementzis, 2000; Vermeiren, Devlieghere, De Graef, & Debevere, 2005). Many authors and quality control of food industries have set LAB concentration of  $10^7$  CFU/g as a criterion for determining meat products shelf-life (Karabagias, Badeka, & Kontominas, 2011; Kreyenschmidt et al., 2010; Slongo et al., 2009).

The use of natural antimicrobial agents as preservatives to extend the shelf-life of foods has been increased due to consumer's demand for more natural ingredients and less chemical additives (Calo, Crandall, O'Bryan, & Ricke, 2015; Petrou, Tsiraki, Giatrakou, & Savvaidis, 2012). Essential oils are produced as secondary metabolites by aromatic plants, they are volatile and their natural complex compounds are characterized by phenolic components (Burt, 2004). They are known for their antimicrobial activity and have been used as natural preservatives to increase food products shelf-life (Teixeira et al., 2013). Oregano essential oil (OEO) is considered one of the most effective among the essential oils due to its antimicrobial action (Aguirre, Borneo, & León, 2013; Emiroğlu, Yemiş, Coşkun, & Candoğan, 2010). Many studies have used OEO as antimicrobial agent against spoilage and pathogenic

microorganisms in meat and meat products (Frangos, Pyrgotou, Giatrakou, Ntzimani, & Savvaidis, 2010; Goulas & Kontominas, 2007; Hasapidou & Savvaidis, 2011; Jouki, Yazdia, Mortazavia, Koocheki, & Khazaei, 2014; Mexis, Chouliara, & Kontominas, 2009).

Mathematical modeling is an important tool to describe food shelflife (Koutsoumanis & Nychas, 2000; Mataragas, Drosinos, Vaidanis, & Metaxopoulos, 2006). The temperature is one of the most important environmental parameters, from food safety and quality points of view (Gospavic, Kreyenschmidt, Bruckner, Popov, & Haque, 2008). Growth parameters (maximum specific growth rate and lag phase) are highly temperature dependent, and temperature increase (below the optimal) tends to reduce the food shelf-life and quality (Cayré, Vignolo, & Garro, 2003; Longhi, Dalcanton, De Aragão, Carciofi, & Laurindo, 2013).

The aim of the current study was to model (primary and secondary mathematical models) the effect of OEO as an antimicrobial agent on LAB natural microbiota growth in vacuum-packed cooked sliced ham stored at different temperatures.

# 2. Material and methods

## 2.1. Sample preparation

Five cooked ham pieces (about 3 kg) (Seara®, São Paulo, Brazil)

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<sup>\*</sup> Corresponding author. E-mail addresses: ealdaniel@ufpr.br (D.A. Longhi), glaucia.aragao@ufsc.br (G.M.F. de Aragão).

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were acquired in local markets, stored at 4 °C and prepared aseptically in a slicer (Metvisa, model CFIE 250, Brusque, Brazil), with thick set to 1.5 mm and approximate total mass of 20 g per slice. Each cooked ham piece (from different production batch) was used for one isothermal experiment. The samples were divided into two groups, control (without OEO) and with OEO (0.4% of oregano (Origanum vulgare) essential oil) (Inter-link LTDA, Jandira, São Paulo). The OEO was applied at the samples surface. For each sample, OEO was applied using a micropipette to achieve 0.4% (v/w) final concentration. Samples (about 20 g) were put into sterile homogenization bags and placed in an incubator with temperature control (Dist. Florianopolis, Brazil). Five different temperatures were tested to reflect the different storage conditions of refrigerated foods, from market to home. The temperature of 6 °C was chosen based on Worsfold and Griffith (1997) and Evans and Redmond (2015) that considered this is the average temperature of domestic refrigerators. Worsfold and Griffith (1997), Staskel, Sweitzer, Briley, Roberts-Gray, and Almansour (2009) and Geppert, Reger, Bichler, and Stamminger (2010) considered that 12 °C is the maximum temperature of domestic refrigerators. The temperature abuses (15 and 20 °C) and the ambient temperature (25 °C) were chosen based on Geppert et al. (2010). These conditions could reflect retail display, the temperature increase during the time spent in store and in a car/ transport to home during warmer months. The temperature around the samples was recorded by data-loggers (Testo 174, Lenzkirch, Germany) every 10 min. The microbial growth was measured in duplicate by plate count method (concentration expressed in CFU/g) until the stationary growth phase.

Measurements of pH, water activity and sodium chloride were carried out to verify the physicochemical composition. pH was measured by using portable digital pH-meter model 205 (TESTO, Sparta, USA). Water activity ( $a_w$ ) was measured with a dew-point hygrometer (Aqualab, SERIES 3TE, Pullman, USA). Sodium chloride was determined as proposed by Aliño, Fuentes, Fernández-Segovia, and Barat (2011). Homogenization samples were made up to 100 mL and then centrifuged (Sigma, 4 k15 model) during 10 min at 4000 rpm to remove any fine debris present in the sample. An aliquot of the supernatant obtained was taken, filtered and a sample of exactly 500 µL aliquot was titrated by using Chloride Analyzer equipment (Cole Parmer, 926 model). The results were expressed in milligrams of Chlorine per liter of solution.

## 2.2. Microbiological analysis

All ham samples were diluted in peptone water (1% w/v) in the ratio 9:1 [volume peptone water (mL): ham mass (g)] in homogenization packages. Homogenization was performed for 60 s in a stomacher (ITR model 1204) to carry out the first dilution. The following dilutions were performed in test tubes containing peptone water (1% w/v). Then, 1 mL of each dilution was transferred to sterile Petri dishes with double layer of agar MRS (pH 6.5  $\pm$  0.2) broth (Difco Laboratories, Detroit, USA). All the procedure was carried out in laminar flow chamber. The inverted plates were incubated at 30 °C for 48 h. The LAB concentration was expressed in CFU/g of ham.

#### 2.3. Growth parameters estimation

The experimental data of the LAB growth were transformed to log (CFU/g) for the fitting procedure. Baranyi and Roberts (1994) (Eqs. (1) and (2)) and modified Gompertz (Zwietering, Jongenburger, Rombouts, & Vant Riet, 1990) (Eq. (3)) primary models, that describe the logarithm of the microbial concentration (y = log(N) or  $Y = log(N/N_0)$ ) as function of the time (t), were fitted to the experimental growth data of LAB in vacuum-packed cooked sliced ham for the control samples and samples with 0.4% OEO. The fitting procedure was performed in Matlab R2013a (MathWorks<sup>®</sup>, Natick, USA), in which the following growth parameters were estimated:  $h_0$  (related to the physiological

state of cells),  $\mu_{max}$  (maximum specific growth rate),  $y_0 = log(N_0)$ (logarithm of initial microbial concentration),  $y_{max} = log(N_{max})$  (logarithm of maximum microbial concentration),  $\lambda$  (duration of lag phase) and *A* (amplitude of microbial growth concentration, i.e.,  $A = y_{max}-y_0$ ). To obtain the best fitting of Baranyi and Roberts model, the estimation was performed in two steps. In the first step, the estimation of the parameters was carried out by fitting the model to the experimental data. The arithmetic mean value of  $h_0$  parameter for every temperature was calculated. In the second step, the  $h_0$  parameter was set with the calculated mean value, and  $\mu_{max}$ ,  $y_0$  and  $y_{max}$  were estimated again by the new fitting. The parameter  $\lambda$  of Baranyi and Roberts model can be obtained with the Eq. (4).

$$y = y_0 + \mu_{max}F(t) - \ln\left(1 + \frac{e^{\mu_{max}F(t)} - 1}{e^{y_{max}-y_0}}\right)$$
(1)

$$F(t) = t + \frac{1}{\mu_{max}} \ln(e^{(-\mu_{max}t)} + e^{(-h_0)} - e^{(-\mu_{max}t - h_0)})$$
(2)

$$Y = A \exp\left(-\exp\left(\frac{e\mu_{max}}{A}(\lambda - t) + 1\right)\right)$$
(3)

$$h_0 = \mu_{max} \lambda \tag{4}$$

The Square Root (Ratkowsky, Olley, McMeekin, & Ball, 1982) and the Exponential secondary models (Eqs. (5) and (6), respectively) were used to describe the dependence of  $\mu_{max}$  parameter with the temperature. The fitting procedure was performed in Matlab R2013a (The MathWorks Inc.<sup>\*</sup>, Natick, USA).

$$\sqrt{\mu_{max}} = b(T - T_{min}) \tag{5}$$

$$\mu_{max} = a \, \exp(cT) \tag{6}$$

# 2.4. Statistical analysis

The following statistical indexes were used to obtain the performance of the models: Coefficient of Determination ( $\mathbb{R}^2$ ), Root Mean Square Error (RMSE), Bias factor (BF) and Accuracy factor (AF) (Ross & McMeekin, 1994), shown in Eqs. (7), (8), (9) and (10), respectively, in which *n* is the number of experimental data, *p* is the number of model parameters, *y* are the values of microbial cell concentration,  $\overline{y}$  is the arithmetic mean of all values of *y*, *exp* are the values obtained in the experiments, and *pred* are the values predicted by the model. The 95% confidence interval of model parameters obtained in the fitting procedure in Matlab were also analyzed.

$$R^{2} = \frac{\sum (v_{pred} - \overline{y})^{2}}{\sum (v_{exp} - \overline{y})^{2}}$$
(7)

$$RMSE = \sqrt{\frac{\sum (y_{exp} - y_{pred})^2}{n - p}}$$
(8)

 $bias factor = 10^{\left(\sum \frac{\log(y_{pred}/y_{exp})}{n}\right)}$ (9)

$$accuracy factor = 10^{\left(\sum \frac{|\log(y_{pred}/y_{exp})|}{n}\right)}$$
(10)

# 3. Results and discussion

The physicochemical analyzes were performed in triplicate for each piece of ham used in the experiments. The average values ( $\pm$  standard error) of pH = 6.22 ( $\pm$  0.04), water activity = 0.970 ( $\pm$  0.001) and sodium chloride (% in mass) = 2.88% ( $\pm$  0.57) were found in the physicochemical analysis of the samples. The low standard errors observed in the results guarantee that the samples were very similar in composition.



**Fig. 1.** Growth curves of LAB natural microbiota at 6  $^{\circ}$ C (a), 12 and 15  $^{\circ}$ C (b), and 20 and 25  $^{\circ}$ C (c) of control samples (filled symbols) and samples with 0.4% OEO (unfilled symbols), and the fitting of Baranyi and Roberts (continuous lines) and modified Gompertz (dashed lines) models to the experimental data obtained in ham.

In the first step, Baranyi and Roberts model (BAR) was fitted to the experimental data of the LAB growth in vacuum-packed cooked sliced ham under five different isothermal conditions (6, 12, 15, 20 and 25 °C ( $\pm$  0.5)) and the four model parameters ( $h_0$ ,  $\mu_{max}$ ,  $y_0$  and  $y_{max}$ ) were estimated. As proposed by various authors, e.g. Baranyi, Robinson, Kaloti, and Mackey (1995), Amézquita, Weller, Wang, Thippareddi, and Burson (2005) and Gospavic et al. (2008), the average value

( $\pm$  standard deviation) for parameter  $h_0$  was calculated, resulting in  $h_0 = 8.44$  ( $\pm 2.58$ ). In the second step, the Baranyi and Roberts model (with fixed value of  $h_0$  parameter) was fitted again to the experimental data of the LAB growth in ham. LAB concentration of  $10^7$  CFU/g (7 log CFU/g) was considered as criterion to determine ham shelf-life, as shown in Fig. 1. The fitting of modified Gompertz primary model to the isothermal experimental data are also shown in Fig. 1. According to

#### Table 1

Temperature (°C)	Model	Sample	$\mu_{max}$ (d <sup>-1</sup> )	λ <sup>a</sup> (d)	A or $y_{max}$ (log CFU/g) <sup>b</sup>	Shelf-life (d)
6	BAR	Control	0.79 ( ± 0.11)	10.75 ( ± 2.08)	8.73 ( ± 0.93)	15.36
		OEO	0.65 ( ± 0.10)	13.0 ( ± 2.28)	5.52 ( ± 0.33)	> 45 <sup>c</sup>
	GOM	Control	0.49 ( ± 0.44)	7.18 ( ± 6.27)	6.45 ( ± 3.10)	15.48
		OEO	0.20 ( ± 0.17)	6.30 ( ± 7.1)	2.64 ( ± 0.47)	> 45 <sup>c</sup>
12	BAR	Control	$1.61 (\pm 0.16)$	5.23 ( ± 0.78)	7.79 ( ± 0.40)	8.77
		OEO	0.96 ( ± 0.12)	8.82 ( ± 1.63)	8.39 ( ± 1.62)	15.84
	GOM	Control	0.89 ( ± 0.23)	3.59 ( ± 0.89)	5.58 ( ± 0.26)	9.47
		OEO	$1.10(\pm 0.71)$	9.20 ( ± 2.09)	8.10 ( ± 3.28)	13.37
15	BAR	Control	$1.67 (\pm 0.18)$	5.10 ( ± 0.73)	8.35 ( ± 0.61)	8.36
		OEO	$1.04(\pm 0.25)$	8.15 ( ± 2.95)	8.31 ( ± 2.47)	14.15
	GOM	Control	$1.12(\pm 0.35)$	$3.98(\pm 0.88)$	6.52 ( ± 0.77)	8.28
		OEO	4.84 ( ± 2.94)	11.37 ( ± 0.44)	6.77 ( ± 0.53)	12.36
20	BAR	Control	4.41 ( ± 0.77)	$1.91 (\pm 0.42)$	8.17 ( ± 0.44)	3.04
		OEO	3.34 ( ± 0.58)	2.53 ( ± 0.73)	8.07 ( ± 0.84)	3.63
	GOM	Control	3.88 ( ± 2.64)	$1.63 (\pm 0.36)$	5.37 ( ± 0.48)	2.83
		OEO	$1.57 (\pm 0.52)$	$1.01 (\pm 0.54)$	6.13 ( ± 1.44)	3.81
25	BAR	Control	7.10 ( ± 0.44)	$1.19(\pm 0.10)$	8.21 ( ± 0.20)	1.80
		OEO	5.84 ( ± 0.67)	$1.45(\pm 0.21)$	7.89 ( ± 0.60)	2.31
	GOM	Control	4.87 ( ± 1.91)	0.87 ( ± 0.20)	5.28 ( ± 0.35)	1.78
		OEO	7.16 ( ± 5.21)	$1.43 (\pm 0.22)$	4.85 ( ± 0.59)	2.08

Growth parameters ( $\pm$  95% confidence interval) estimated by fitting of modified Gompertz (GOM) and Baranyi and Roberts (BAR) models (second step, fixed  $h_0 = 8.44$  ( $\pm$  2.58)) to the experimental data of control samples and samples with 0.4% OEO in ham at 6, 12, 15, 20 and 25 °C and the statistical indexes of the fitting.

<sup>a</sup> Duration of the lag phase was obtained by Eq. (4).

<sup>b</sup> A parameter for GOM model and  $y_{max}$  parameter for BAR model.

<sup>c</sup> The value that defines the end of the shelf-life (10<sup>7</sup> CFU/g) was not reached until the end of the experiment (45 days).

## Table 2

Statistical indexes obtained by the fitting of modified Gompertz (GOM) and Baranyi and Roberts (BAR) models to the experimental data of control samples and samples with 0.4% OEO in ham at 6, 12, 15, 20 and 25  $^\circ$ C.

Temperature	Model	Sample	Statistical indices				
(0)			$\mathbb{R}^2$	RMSE	BF	AF	
6	BAR	Control	0.951	0.633	1.006	1.080	
		OEO	0.873	0.414	1.003	1.067	
	GOM	Control	0.922	1.841	0.987	1.086	
		OEO	0.849	1.038	1.001	1.064	
12	BAR	Control	0.981	0.370	1.002	1.047	
		OEO	0.963	0.650	1.001	1.097	
	GOM	Control	0.995	0.341	0.999	1.011	
		OEO	0.949	1.519	0.950	1.121	
15	BAR	Control	0.988	0.363	1.002	1.044	
		OEO	0.955	0.859	1.003	1.102	
	GOM	Control	0.998	0.337	1.000	1.011	
		OEO	0.994	0.530	0.956	1.046	
20	BAR	Control	0.985	0.364	1.001	1.038	
		OEO	0.944	0.592	1.008	1.096	
	GOM	Control	0.980	0.830	0.986	1.035	
		OEO	0.986	0.676	1.001	1.030	
25	BAR	Control	0.991	0.238	1.001	1.033	
		OEO	0.968	0.451	1.003	1.065	
	GOM	Control	0.979	0.830	0.970	1.057	
		OEO	0.970	0.997	0.967	1.067	

Baranyi et al. (1995), the value of  $h_0$  parameter depends only on the initial physiological state of the cells. This value should be the same for all different temperatures (considering the same inoculum and food).

In the experiment at 6 °C, shown in Fig. 1(a), the LAB concentration in the control sample reached the value of 7 log CFU/g at 15<sup>th</sup> day of storage, while LAB concentration in samples with 0.4% OEO did not reach the concentration of 7 log CFU/g until the  $45^{\text{th}}$  day of storage. Thus, at 6 °C, the addition of 0.4% OEO extended the microbiological shelf-life of ham for, at least, 30 days, when was compared with the control. Note that the OEO was effective in extending the ham shelf-life, and its application reduced the LAB concentration by 3.0 log cycles when compared to control sample. Chouliara, Karatapanis, Savvaidis, and Kontominas (2007) analyzed the effect of OEO at concentrations of 0.1 and 1% (v/w) in fresh chicken stored at 4 °C. The use of OEO (0.1%) resulted in a decrease of 1 log CFU/g, when compared with the control samples that reached the concentration of 7 log CFU/g in 9 days of storage. The concentration of 1% OEO completely inhibited the growth of LAB until 12 days of storage and extended product shelf-life for 16 days. Ntzimani, Giatrakou, and Savvaidis (2010) observed an extension in the shelf-life of precooked chicken meat packaged under vacuum and with the addition of 0.2% OEO for 6 days in the control. Mexis et al. (2009) observed the effect of 0.1% OEO in tarama salad stored at 4 °C. The control samples reached the concentration of 6.45 log CFU/g at 24<sup>th</sup> day of storage and, in the product with OEO, the LAB concentration reached 5.95 log CFU/g at 40<sup>th</sup> day of storage.

In the experiment at 12 °C (temperature abuse, considering refrigeration conditions), shown in Fig. 1(b), LAB concentration reached

#### Table 3

Estimated parameter values (  $\pm$  confidence interval) (*a*, *b*, *c* and  $T_{min}$ ) of the fitting of Square Root and Exponential secondary models to  $\mu_{max}$  parameter values and the statistical indexes (R<sup>2</sup> and RMSE).

Primary model	Sample	Square root				Exponential			
		$b (^{\circ}C^{-1}h^{-0.5})$	<i>T<sub>min</sub></i> (°C)	$\mathbb{R}^2$	RMSE	a (h <sup>0.5</sup> )	с	$\mathbb{R}^2$	RMSE
BAR	Control OEO	$0.0958 (\pm 0.0457)$ $0.0886 (\pm 0.0588)$	$-1.53 (\pm 8.71)$ -0.31 (± 11.41)	0.937 0.884	0.210 0.270	$0.361 (\pm 0.323)$ $0.190 (\pm 0.223)$	$0.120 (\pm 0.039)$ $0.138 (\pm 0.050)$	0.982 0.980	0.408 0.365
GOM	Control OEO	$0.0875 (\pm 0.050)$ $0.0998 (\pm 0.1330)$	$-0.12 ( \pm 9.63)$ $0.32 ( \pm 22.17)$	0.914 0.655	0.227 0.611	$0.296 (\pm 0.591)$ $0.390 (\pm 1.836)$	$\begin{array}{l} 0.114 \ (\ \pm \ 0.087) \\ 0.113 \ (\ \pm \ 0.205) \end{array}$	0.912 0.623	0.678 2.07



Fig. 2. Fitting of (a) Square Root and (b) Exponential secondary models (continuous lines) to  $\mu_{max}$  parameter of control samples (filled symbols) and samples with 0.4% OEO (unfilled symbols) data.

7 log CFU/g at  $15^{\text{th}}$  day of storage, 7 days longer as compared to the control sample at the same temperature. The shelf-life of ham at  $15 \,^{\circ}$ C, shown in Fig. 1(b), was 14 days for samples with 0.4% OEO and 8 days for control samples. In the experiments at 20 and  $25 \,^{\circ}$ C, shown in Fig. 1(c), the shelf-life of ham for control and OEO samples were similar. It was observed that antimicrobial activity of OEO is higher at lower storage temperatures. Costa (2013) evaluated the effect of thermochemical treatment (OEO and heat) in *Perna perna* mussels and found that, with increasing storage temperature, there was a decrease in the efficiency of the treatment.

Table 1 shows the values of the growth parameters ( $\pm$  95% confidence interval) estimated by fitting of Baranyi and Roberts, and modified Gompertz models to the experimental data obtained in ham at 6, 12, 15, 20 and 25 °C to the control samples and samples with 0.4% OEO. The estimated parameters of Baranyi and Roberts model presented lower values of 95% confidence interval than parameters of modified Gompertz in most cases.

The storage temperature had a great influence on growth parameters ( $\lambda$ ,  $\mu_{max}$  and  $y_{max}$ ). Analyzing the results of Baranyi and Roberts model, the value of  $\mu_{max}$  parameter increased almost nine times from 6 °C to 25 °C (from 0.79 day<sup>-1</sup> to 7.10 day<sup>-1</sup> in control samples, and from 0.65 day<sup>-1</sup> to 5.84 day<sup>-1</sup> in samples with 0.4% OEO). The value of  $\lambda$  parameter decreased proportionally almost nine times from 6 °C to 25 °C (from 10.75 day to 1.19 day in control samples, and from 13.00 day to 1.45 day in samples with 0.4% OEO). The average value of the  $y_{max}$  parameter (  $\pm$  standard deviation) found was 8.25 log CFU/g (  $\pm$  0.30) for control samples and 7.64 log CFU/g (  $\pm$  1.07) for samples with 0.4% OEO. In the experiments at 6 °C, the addition of 0.4% OEO resulted in a reduction of 3 log CFU/g on the maximum population ( $y_{max}$ ) (from 8.73 log CFU/g in control sample to 5.52 log CFU/g in sample with 0.4% OEO). Other authors found similar values of  $y_{max}$  parameter. Kreyenschmidt et al. (2010) analyzed the shelf-life of vacuum-packed cooked sliced ham and found  $y_{max}$  values between 7.8 and 8.7 log CFU/g. Liu et al. (2012) found values between 7 and 8 log CFU/g in the LAB growth in vacuum-packed sliced ham. Mataragas et al. (2006) assessed the deteriorating microbiota of sliced meat products and found values of maximum population between 8.3 and 8.9 log CFU/g.

The statistical indexes ( $\mathbb{R}^2$ , RMSE, Bias factor and Accuracy factor) obtained by the fitting of Baranyi and Roberts, and modified Gompertz models to the experimental data are shown in Table 2. Both mathematical models fitted well to the experimental data, however, the Baranyi and Roberts model showed better results of  $\mathbb{R}^2$ , RMSE, bias factor and accuracy factor in most cases. The average  $\mathbb{R}^2$  value for the control samples ( $\pm$  standard deviation) was 0.979 ( $\pm$  0.014) and for samples with 0.4% OEO was 0.941 ( $\pm$  0.035) for Baranyi and Roberts model. For modified Gompertz, the average  $\mathbb{R}^2$  value for the control samples was 0.975 ( $\pm$  0.027) and for samples with 0.4% OEO was 0.950 ( $\pm$  0.055). Slongo et al. (2009) obtained average  $\mathbb{R}^2$  value of 0.80 evaluating the growth of LAB in pressurized hams. Gospavic et al. (2008) studied the growth of *Pseudomonas* spp. in chicken meat at different temperatures, finding  $\mathbb{R}^2$  values around 0.98 for the Baranyi

and Roberts model. Kreyenschmidt et al. (2010) found R<sup>2</sup> values from 0.93 to 0.98 for determining the shelf-life of cooked sliced ham based on the growth of LAB. The average RMSE value ( $\pm$  standard deviation) for the control samples was 0.394 (  $\pm$  0.130) and for samples with 0.4% OEO was 0.593 (  $\pm$  0.159) for Baranyi and Roberts model. For modified Gompertz, the average RMSE value for the control samples was 0.836 (  $\pm$  0.548) and for samples with 0.4% OEO was 0.952  $(\pm 0.342)$ . Krevenschmidt et al. (2010) found RMSE values ranging from 0.409 to 0.800 for natural microbiota. These R<sup>2</sup> and RMSE values are acceptable once the microbial concentrations are from natural microbiota of solid food, which can lead to changes in scores. The values of Bias factor and Accuracy factor found were close to 1, indicating that the observed response is as close as the predicted response. The average Accuracy factor value (  $\pm$  standard deviation) for the control samples was 1.048 (  $\pm$  0.017) and for samples with 0.4% OEO samples was 1.085 (± 0.016) for Baranyi and Roberts model. For modified Gompertz, the average Accuracy factor value for the control samples was 1.040 (  $\pm$  0.029) and for samples with 0.4% OEO samples was 1.066  $(\pm 0.031).$ 

The estimated parameter values (  $\pm$  95% confidence interval) (*a*, *b*, c and  $T_{min}$ ) of the fitting of Square Root and Exponential secondary models to  $\mu_{max}$  parameter values and the statistical indexes (R<sup>2</sup> and RMSE) are shown in Table 3, and the fitting of secondary models to  $\mu_{max}$ parameter of control samples and samples with 0.4% OEO are shown in Fig. 2. The fitting of the Exponential model to the data of  $\mu_{max}$  parameter of Baranyi and Roberts model was better than the fitting of Square Root model for both samples (control and OEO), as verified by higher R<sup>2</sup> and smaller RMSE values. On the other hand, both secondary models did not fit well to the data of  $\mu_{max}$  parameter of modified Gompertz for both samples (control and OEO), as verified by lower R<sup>2</sup> and higher RMSE values. Thus, the Baranyi and Roberts primary model with Exponential secondary model can be used to predict the LAB growth in vacuum-packed cooked sliced ham under non-isothermal conditions (temperature variations during the production, storage and distribution) for both control samples and samples with 0.4% OEO.

OEO has proved to be useful as food antimicrobial. However, its applications for direct consumption can be compromised by their strong sensorial characteristics. Cooked ham with addition of OEO can be used, for example, for ready-to-eat pizzas or products based on oreganoflavored ham. Further studies on sensorial acceptance of ham with OEO are needed.

#### 4. Conclusion

Baranyi and Roberts, and modified Gompertz models were able to describe the growth of LAB natural microbiota in vacuum-packed cooked sliced ham, as verified by the statistical indexes  $R^2$ , RMSE, bias factor and accuracy factor. The fitting of Baranyi and Roberts model resulted in better statistical indexes in most cases and lower 95% confidence interval of parameters. The value of  $\mu_{max}$  parameter increased almost nine times from 6 °C to 25 °C. The use of the essential oil led to an increase of  $\lambda$ , a decrease of  $\mu_{max}$  and a consequent increase in the ham shelf-life in relation to control samples. This study showed clearly that the addition of oregano essential oil enhanced the shelf-life of vacuum-packed cooked sliced ham based on LAB population (at 6 °C, the shelf-life was extended for, at least, 30 days when compared to control). Thus, the results indicated that the oregano essential oil could be used as natural antimicrobial agent in ham in different applications of the food industry.

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